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Development of a Two-Step Enrichment Procedure for Recovery of *Yersinia enterocolitica* from Food

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Two new enrichment media were formulated for the recovery of *Yersinia enterocolitica* from foods: (i) yeast extract-rose bengal broth for preenrichment at 4 or 10°C; and (ii) bile-oxalate-sorbose broth, a selective enrichment incubated at 22°C. Comparison of these media in a two-step enrichment procedure against cold enrichment and modified Rappaport broth showed improved and more rapid recovery of human strains of *Y. enterocolitica* from inoculated foods. The use of bile-oxalate-sorbose broth as a selective enrichment also improved the performance of cold enrichment with phosphate-buffered saline. Determination of the best enrichment system for recovery of *Y. enterocolitica* from samples of retail pork and fresh pork tongues depended on whether the criterion was the number of positive samples, the variety of different serotypes recovered, or the ability to recover the important human serotype O:3. A single enrichment system with the widest selectivity would include preenrichment at 4°C with either phosphate-buffered saline for 14 days or yeast extract-rose bengal broth for 9 days followed by selective enrichment with bile-oxalate-sorbose broth at 22°C for 5 days.

Yersiniosis has often been described as a food-borne disease (13, 15), a deduction supported by descriptions of outbreaks in which some common vehicle was likely (1, 16) and by the fact that a food animal, swine, is a reservoir of the common serotype O:3. Despite outbreaks suggesting a common vehicle and a recognized food animal reservoir, there has been to date only one outbreak in which the vehicle was clearly identified. That outbreak occurred in New York in 1976, and the vehicle was chocolate milk. The milk contained the same serotype, O:8, as was isolated from ill individuals (5). The inability to identify vehicles in other outbreaks may be due in part to unreliable laboratory techniques for recovery of pathogenic forms of *Yersinia enterocolitica*.

Laboratory methods first used for recovery of *Y. enterocolitica* from foods were based primarily on cold enrichment techniques previously described for examination of feces (7, 8), and there have been few improvements since. Isolation of *Y. enterocolitica* from food is more difficult than from feces taken from patients with active infections in that the numbers of *Yersinia* may be smaller, and the background flora is likely to be greater in both number and variety. Pai et al. (17) have concluded that cold enrichment is not necessary for recovery of *Y. enterocolitica* from stool specimens taken from patients with symptoms; and van Nøyen et al. (26) suggested that the additional recoveries obtained by cold enrichment were types of *Y. enterocolitica* that were not clinically important. In the case of foods, however, enrichment provides the only certain way for selecting out low numbers of *Yersinia* from the total microbial population.

Phosphate-buffered saline (PBS) has been a common cold enrichment medium for both feces and foods. Mehlman et al. (14) found that supplementation of PBS with 1% sorbitol and 0.15% bile salts gave better results. Vidon and Delmas (28) used a phosphate buffer supplemented with Pastone, sodium chloride, and cycloheximide for cold enrichment of raw milk samples. Schiemann (20) and Hanna et al. (9) used PBS supplemented with 1% mannitol, previously described by Christenson for stool specimens (E. H. Christenson and G. P. Jansen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C43, p. 42), for cold enrichment of foods. van Pee and Stragier (27) suggested that a rich broth such as trypticase soy is preferred for cold enrichment.

Because of the long time period required for cold enrichment, there have been various efforts to devise selective enrichments which could be incubated for shorter times at higher temperatures. Modified Rappaport broth was first described by Wauters (29; G. Wauters, Ph.D. thesis, University of Louvain, Vander, Belgium, 1970), and was used at 22°C for 2 days or longer.

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This highly selective medium is excellent for the recovery of serotype O:3 if the inoculum is sufficient, but is inhibitory to certain other serotypes, especially O:8 (11, 12, 20). Inoue and Kurose (10) used selenite medium with novobiocin at 37°C with less success than cold enrichment. Lee et al. (12) described two modified selenite media incubated at 22°C that were effective for recovery of certain strains of *Y. enterocolitica*, but only when the food sample was limited to 0.2 g in 100 ml of enrichment broth.

There have been only a few attempts to devise two-step enrichment techniques for recovery of *Y. enterocolitica* from foods. Schiemann and Toma (23) found that the use of modified Rappaport broth after cold enrichment significantly increased the recovery of *Y. enterocolitica* from raw milk. Schiemann (21) subsequently found a lower recovery rate from raw pork by this two-step method compared to modified Rappaport broth used alone at 22°C for 7 days, which may have been due to a shorter incubation time used for pork samples (2 days) than with raw milk (5 days). Vidon and Delmas (28) also obtained greater recoveries from raw milk by using a selective enrichment of their own formulation incubated at 28°C for 48 h after cold enrichment for 1 month.

This paper reports the evaluation of a new selective enrichment for the recovery of *Y. enterocolitica* from foods and its usefulness in combination with certain preenrichment media.

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used in this paper: PBS, phosphate-buffered saline; PSB, PBS-sorbitol-bile salts; MRB, modified Rappaport broth without carbenicillin; YER, yeast extract-rose bengal; YEN, YER plus NaCl; BOS, bile-oxalate-sorbose; BON, BOS plus NaCl; TSYE, Trypticase soy-yeast extract; CIN, cefsulodin-irgasan-novobiocin; KIA, Kligler iron agar.

Enrichment media. Standard enrichment media which have been described and used previously by others and selected for comparative purposes in this study included: (i) PBS M/15 (pH. 7.6) with 1.0% sorbitol and 0.15% bile salts (14) (PSB) incubated at 4°C for 14 days; and (ii) modified Rappaport broth (Wauters, Ph.D. thesis) without carbenicillin (MRB) incubated at 22°C. Carbenicillin was deleted from modified Rappaport broth after studies showed that this antibiotic inhibited growth of certain strains of *Y. enterocolitica* O:3, for which this medium is specifically designed, and that the high selectivity of the medium was only slightly lowered without the antibiotic. Aging of the medium reduced the inhibitory properties of the antibiotic when it was present, which may account for the variable performance sometimes observed with this medium. PSB and MRB were used for direct enrichment, PSB was used for preenrichment, and MRB was used for a secondary selective enrichment.

An experimental preenrichment broth, designated YER broth, was formulated containing (per liter): $Na_2HPO_4 \cdot 7H_2O$, 17.25 g; yeast extract, 5.0 g; bile salts (Difco Laboratories), 2.0 g; NaCl, 1.0 g (10 ml × 10%); and $MgSO_4 \cdot 7H_2O$, 0.01 g (10 ml × 0.1%). These ingredients were dissolved in 770 ml of distilled water, and the solution was adjusted to pH 7.9 with 5 N HCl and then autoclaved at 121°C for 15 min. The following filter-sterilized solutions were added to the cooled sterile basal (per liter): sorbose, 100 ml × 4.0%; pyruvic acid (sodium salt), 100 ml × 1.0%; rose bengal, 10 ml × 4.0 mg/ml. The final pH was adjusted to 7.9 with either 5 N NaOH or HCl as required. This preenrichment broth was incubated at either 10 or 4°C. A modification of the medium, YEN, was prepared by adding 4.0% sodium chloride and was incubated at 22°C only.

An experimental selective enrichment medium designated BOS broth was formulated with (per liter): $Na_2HPO_4 \cdot 7H_2O$, 17.25 g; sodium oxalate, 5.0 g; bile salts (Difco), 2.0 g; NaCl, 1.0 g (10 ml × 10%); $MgSO_4 \cdot 7H_2O$, 0.01 g (10 ml × 0.1%). These ingredients were dissolved in 639 ml of distilled water, the pH was adjusted to 7.6 with 5 N HCl, and the solution was autoclaved at 121°C for 15 min. The following filter-sterilized solutions were then added (per liter): sorbose, 100 ml × 10%; asparagine, 100 ml × 1.0%; methionine, 100 ml × 1.0%; metanil yellow, 10 ml × 2.5 mg/ml; yeast extract, 10 ml × 2.5 mg/ml; pyruvic acid (sodium salt), 10 ml × 0.5%. One milliliter of a 0.4% solution of Irgasan DP300 (2,4,4'-trichloro-2'-hydroxy diphenyl ether; Ciba-Geigy) in 95% ethanol was added before adjusting the pH to 7.6 with either 5 N NaOH or HCl as required. Sodium furadantin (10 ml × 1.0 mg/ml) was added from a frozen stock solution (−70°C) on the day of use. A modification of BOS, BON, was prepared by adding 2.5% NaCl. BOS and BON broths were always incubated at 22°C.

Before BOS was evaluated with inoculated and natural foods, it was examined with 27 pure cultures of *Y. enterocolitica* that represented nine different serotypes. All cultures grew in BOS from an inoculum of either 10^4 (15 cultures) or 10^2 (12 cultures) cells. The concentration of NaCl used in formulating BON broth was determined by titration against 15 strains that represented six serotypes of *Y. enterocolitica*. All strains grew well from an inoculum of 10^4 cells in 2.5% NaCl. BON was further evaluated with eight additional cultures of serotype O:5,27 to verify the beneficial effect of NaCl for growth of this particular serotype in this medium. The selective properties of both BOS and BON broths were examined with a number of pure cultures of other gram-negative bacteria.

All preenrichment media were inoculated at a ratio of 10 g of food sample to 90 ml of broth (1:10). Selective enrichments were inoculated with 1.0 ml of preenrichment medium per 100 ml of broth. BOS was evaluated as a single-step enrichment medium, but the introduction of food at a 1:10 ratio overwhelmed its selective properties. Direct isolation from some samples was attempted by inserting a cotton swab directly into the mixed or homogenized sample at multiple points and then streaking onto the selective agar medium. This technique was added to verify the necessity of enrichment for recovery of *Y. enterocolitica* from the foods.

***Y. enterocolitica* cultures.** All cultures of *Y. enteroco-*

litica used in food inoculation studies were originally obtained from S. Toma, Canadian National Reference Service for *Yersinia*. The strains selected had previously shown certain characteristics of virulence (22). Cultures were streaked from a stock slant onto a plate of Trypticase soy (BBL Microbiology Systems)–0.6% yeast extract (TSYE) agar which was incubated at 22°C for 48 h. A suspension was prepared in PBS, and the density was adjusted by comparison to a McFarland standard. A viable cell count was completed by plating suitable dilutions onto TSYE agar.

Inoculated foods. The evaluation of the various enrichment systems by recovery of human strains of *Y. enterocolitica* inoculated into raw foods (ground meats, celery) was consistently frustrated by the presence of endogenous strains of *Y. enterocolitica*. The experimental approach for this purpose was then changed to the use of commercially "sterile" food products to which was added an "artificial" background composed of a mixture of five (one experiment) or six gram-negative bacteria selected from this group of laboratory cultures: *Aeromonas hydrophila, Enterobacter agglomerans, Serratia liquefaciens, Pseudomonas fluorescens, Citrobacter freundii, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas putida, Proteus morganii, Enterobacter cloacae, Klebsiella pneumoniae*, and *Enterobacter aerogenes*. All of these cultures were originally isolated from foods, with the exception of *A. hydrophila* and *K. pneumoniae* from water, *E. coli* from feces, and *P. fluorescens* and *P. aeruginosa* from hospital environmental swabs. These organisms were selected because they were found during developmental studies to represent the most difficult to control with selective agents, frequently outgrew and inhibited the growth of *Y. enterocolitica*, and were not always inhibited on the isolation agar (CIN). Consequently, they provided the most vigorous challenge to the enrichment systems for the selection of *Y. enterocolitica*. The cultures were grown on TSYE agar overnight at 32°C. Suspensions were prepared as described above for *Y. enterocolitica*. The background organisms were mixed in equal proportions and added at either 10^6 or 10^5 cells per g of food. One test strain of *Y. enterocolitica* was added to the food at either 10^4 or 10^2 cells per g, providing ratios of background to *Y. enterocolitica* of about 10^2 and 10^4 or 10^1 and 10^3. Organisms were inoculated into the food after preparation and dispensing of the homogenate.

Natural foods. Two groups of natural foods were collected for the final evaluations of certain enrichment systems: (i) 10 samples of raw pork from retail sources, consisting of four samples of ground pork and six samples of pork tongues; and (ii) 20 tongues collected from freshly slaughtered swine. Tongues were prepared for examination by cutting off surface portions from the rear and side and homogenizing the pieces in a blender. Pork was selected because it was a food that would challenge the enrichment systems since it was likely to possess a varied background flora and had the greatest probability for containing important human types of *Y. enterocolitica*.

Isolation agar. All enrichments were streaked onto CIN agar, which was incubated at 32°C for about 20 h. This medium was modified from the original description (19) by substituting sodium deoxycholate at 0.05% for bile salts and reducing the novobiocin concentration to 2.5 mg/liter. Plates inoculated from enrichments for inoculated foods were compared with control plates streaked with a pure culture of the test organism. Colonial morphology under a stereomicroscope with transmitted light was usually distinctive enough to verify the identification of *Y. enterocolitica* among colonies of any background organisms growing on the agar. If there was any doubt, however, about identification, a few colonies were transferred to TSYE agar, and, after incubation, the serotype of the isolate was confirmed by slide agglutination with specific antiserum against the test strain.

Enrichments of natural foods were also streaked onto CIN agar. Plates were examined after incubation at 32°C for about 20 h for colonies resembling *Y. enterocolitica*. Each colonial type present was selected with no fewer than two nor more than four colonies per plate used to individually inoculate KIA slants. KIA slants were incubated overnight at 35°C. Isolates giving typing reactions (K/A^-) were subjected to 22 additional biochemical tests to confirm identification as *Y. enterocolitica* (3) or the related species that have recently been described, *Yersinia intermedia* (6), *Yersinia frederiksenii* (25), and *Yersinia kristensenii* (4). One isolate of each different biotype obtained by each enrichment method and identified as *Y. enterocolitica* was serotyped by using 57 specific antisera prepared against heat-stable antigens in rabbits.

Enrichments of natural foods streaked onto CIN agar provided an opportunity for evaluating the specificity of this isolation medium. For this purpose, isolates that had been selected from CIN agar because they resembled *Y. enterocolitica* in colonial morphology but were biochemically different were further identified by the use of API 20E strips (Analytab Products, Plainview, N.Y.).

RESULTS

Recovery from inoculated foods. In the first experiment presented, two strains each of serotypes O:3 (E675, E676), O:8 (E653, E661), and O:5,27 (E655, E657) were inoculated at two densities in a food containing five different gram-negative bacteria at a density of 1.3×10^5/g (Table 1). The results show that the selective enrichment BOS improved recovery after cold enrichment with PSB for strains of serotype O:8 and O:3, but did not for the two strains of serotype O:5,27. MRB after PSB recovered only one strain of serotype O:3 and the two strains of O:5,27 at the higher density. The use of YER as a preenrichment medium with incubation at 10°C for just 3 days gave better recovery than did PSB. The performance of BOS was improved by extending the incubation time at 22°C from 3 to 5 days. The second experiment presented used the same test strains of *Y. enterocolitica*, but the number of gram-negative organisms in the background was increased to six (Table 2). The advantage of BOS as a selective enrichment over MRB was again demonstrated. YER at 10°C for 3 days with BOS as the selective enrichment gave better recovery when

the incubation of BOS was extended to 5 days. MRB was not useful as a selective enrichment after YER, but its specificity for serotype O:3 strains when used directly was clear. The addition of 4.0% NaCl to YER (YEN) was not productive with incubation at 32°C, but it was successful with incubation at 22°C. This enrichment system (YEN-BOS) was the only one able to recover strain E675 (O:3), with the exception of MRB and strain E657 (O:5,27) at the lower cell density.

In the third experiment a new set of *Y. enterocolitica* strains representing the same serotypes was selected for inoculating the food (Table 3). The background flora was varied slightly in the species included and was increased to 2.2×10^6/g of food. This experiment compared BOS and BON as selective enrichments with three different preenrichments, plus the effect of incubation time on performance of the selective enrichment. PSB-BOS with incubation of BOS for 5 days was the most successful system, demonstrating recovery of all strains at both cell densities. The value of increasing the incubation time for BOS beyond 3 days was clearly evident. The YEN-BOS system failed completely in this experiment in comparison to the relatively good performance in the previous experiment. The erratic performance of YEN may reflect relative differences in salt tolerance of both *Y. enterocolitica* strains and background organisms. The PSB-BOS system, of course, requires the longest time for recovery, i.e., 19 days.

The fourth experiment presented compared BOS and BON as selective enrichments by using three different preenrichments (Table 4). The value of sodium chloride in BOS (BON broth) was apparent. The use of BON as a selective enrichment also reduced background growth on the isolation agar. There was only a slight benefit in increasing the incubation time of YER from 3 to 4 days or of YEN from 2 to 3 days. An attempt to reduce the preenrichment time to 4 h by incubation of YER at 32°C was not successful.

The fifth and sixth experiments used three strains of *Y. enterocolitica* that represent serotypes less commonly isolated from humans but which are sometimes virulent (22). Each of these experiments used a different background flora (Tables 5 and 6). The benefit of BOS after PSB was again apparent. MRB following PSB or YER was less productive; however, MRB used directly was excellent for recovery of strain E768 (O:1,2,3). MRB used directly required only 6 days of incubation at 22°C. BOS after YER at 10°C, requiring 7 to 8 days of incubation, was equivalent to PSB-BOS, which required 17 to 19 days of incubation. The use of BOS after YEN was not a useful combination

TABLE 1. Recovery of *Y. enterocolitica* from vegetables-chicken baby food with an artificial background flora[a]

Preenrichment			Selective enrichment (22°C)		Recovery of *Y. enterocolitica*[b]											
					E676 (O:3)		E675 (O:3)		E661 (O:8)		E653 (O:8)		E655 (O:5,27)		E657 (O:5,27)	
Medium	Temp (°C)	Time (days)	Medium	Time (days)	8.1×10^1[c]	8.1×10^3	1.2×10^2	1.2×10^4	9.3×10^1	9.3×10^3	8.1×10^1	8.1×10^3	7.6×10^1	7.6×10^3	9.3×10^1	9.3×10^3
YER	10	3	BOS	3	++	+++	–	–	+++	+++	+++	+++	–	+	–	+
				4	+++	+++	–	+	+++	+++	+++	+++	–	++	–	++
				5	+++	+++	– (–7d)	+++	+++	+++	+++	+++	+++	+ (+++6d[d])	– (++6d)	+++
PSB	4	14	None		–	–	–	–	–	+++	–	+++	–	+++	–	+++
			BOS	3	+	+	–	–	+++	+++	+++	+++	–	–	–	–
			MRB	3	–	+	–	–	–	–	–	–	–	+	–	+

[a] *A. hydrophila, E. agglomerans, S. liquefaciens, P. fluorescens,* and *C. freundii*; background count, 1.3×10^5/g.

[b] –, No growth; +, separated and countable number of colonies in first streak; ++, confluent growth in first streak and separated colonies in second streak; +++, confluent growth in first and second streaks and separated colonies in third streak.

[c] Number of *Y. enterocolitica* per g of food.

[d] 6d, 6 days.

TABLE 2. Recovery of *Y. enterocolitica* from vegetables-chicken baby food with an artificial background flora[a]

Preenrichment			Selective enrichment (22°C)		Recovery of *Y. enterocolitica*[b] (serotype)											
Medium	Temp (°C)	Time (days)	Medium	Time (days)	E676 (O:3)		E675 (O:3)		E661 (O:8)		E653 (O:8)		E655 (O:5,27)		E657 (O:5,27)	
					1.2×10^{2}[c]	1.2×10^{4}	1.2×10^{2}	1.2×10^{4}	1.2×10^{2}	1.2×10^{4}	1.1×10^{2}	1.1×10^{4}	1.1×10^{2}	1.1×10^{4}	1.1×10^{2}	1.1×10^{4}
YEN	32	3	BOS	3	−	−	−	−	−	+	−	++	−	+	−	−
		4	None		−	−	−	+	−	+	−	+	−	−	−	−
			BOS	3	−	+	−	−	−	+	−	+++	−	−	−	−
YEN	22	3	BOS	3	+	++	+	+++	++	+++	+	++	−	++	−	++
				4	++	ND[d]	+++	ND	ND	ND	++	ND	+	ND	+	ND
YER	10	3	BOS	3	−	+++	−	−	+++	+++	+++	+++	−	−	−	−
				4	+++	ND	−	−	ND	ND	ND	ND	−	++	−	++
				5	ND	ND	−	−	ND	ND	ND	ND	+	ND	−	ND
			MRB	3	−	+	−	−	−	−	−	−	−	+	−	+
				6	+	++	−	−	−	−	−	−	−	−	−	−
MRB	22	6	None		+++	+++	++	+++	−	+	−	−	−	+	−	+
PSB	4	14	None		−	−	−	−	+	++	+	+	−	+++	−	+
			BOS	3	+	++	−	−	+++	+++	+++	+++	−	+	−	+
			MRB	3	+	++	−	−	−	+	−	+	−	−	−	+

[a] *E. agglomerans, E. coli, A. hydrophila, C. freundii, S. liquefaciens*, and *P. aeruginosa*; background count, 1.6×10^{5}/g.
[b,c] See Table 1.
[d] ND, Not done.

TABLE 3. Recovery of *Y. enterocolitica* from beef stew with an artificial background flora[a]

Preenrichment			Selective enrichment (22°C)		Recovery of *Y. enterocolitica*[b] (serotype)											
Medium	Temp (°C)	Time (days)	Medium	Time (days)	E752 (O:3)		E742 (O:3)		E662 (O:8)		E665 (O:8)		E758 (O:5,27)		E654 (O:5,27)	
					1.1×10^{2}[c]	1.1×10^{4}	1.1×10^{2}	1.1×10^{4}	7.3×10^{1}	7.3×10^{3}	1.1×10^{2}	1.1×10^{4}	1.1×10^{2}	1.1×10^{4}	1.1×10^{2}	1.1×10^{4}
YER	10	3	BOS	3	−	+	−	+	+	++	+++	+++	−	−	−	−
				4	−	+++	−	+++	+	ND[d]	ND	ND	−	++	−	−
				5	++	ND	++	ND	++	ND	ND	ND	−	ND	−	−
			BON	3	+	+++	−	+++	−	++	−	+++	−	−	−	−
				4	++	ND	−	ND	−	ND	−	ND	−	−	−	−
				5	ND	ND	+	ND	++	ND	−	ND	−	−	−	−
YEN	22	3	BOS	3	−	−	−	−	−	−	−	−	−	−	−	−
				5	−	−	−	−	−	−	−	−	−	−	−	−
			BON	3	−	+	−	+	−	−	−	−	−	+	−	+
				4	−	+++	+	+++	+	++	−	−	−	+	−	++
				5	+	ND	++	ND	+	ND	−	−	−	ND	−	ND
PSB	4	14	None		−	+	−	+	−	−	−	+++	−	+	−	+++
			BOS	3	+	+	−	++	−	+	+++	+++	+++	+++	−	+
				5	+++	ND	+++	ND	+	ND	ND	ND	+++	+++	+++	+++
			BON	3	+	+	−	+	−	+	++	+++	−	+	+	+
				5	+	+	+	+	−	+	ND	ND	−	−	+	+

[a] *P. putida, P. morganii, E. cloacae, S liquefaciens, E. agglomerans*, and *C. freundii*; background count, 2.2×10^{6}/g.
[b,c] See Table 1.
[d] ND, Not done.

TABLE 4. Recovery of *Y. enterocolitica* from turkey-chicken stew containing an artificial background flora[a]

Preenrichment			Selective enrichment (22°C)		Recovery of *Y. enterocolitica*[b] (serotype)					
					E752 (O:3)		E662 (O:8)		E654 (O:5,27)	
Medium	Temp (°C)	Time (days)	Medium	Time (days)	1×10^{2}[c]	1×10^{4}	3×10^{1}	3×10^{3}	1×10^{2}	1×10^{4}
YER	32	0.25	BOS	2	−	−	−	−	−	−
				3	−	+	+	++	−	−
				5	−	+	−	+++	−	−
			BON	2	−	−	−	−	−	−
				3	−	++	−	−	−	−
				5	+	+++	−	−	+	++
YER	10	3	BOS	3	−	+	−	+++	−	−
				5	+	+++	+++	+++	−	−
									(−7d)[d]	(+++7d)
		4	BOS	3	−	++	+	+++	−	−
				5	++	+++	+++	+++	−	+
									(−6d)	(+++6d)
			BON	3	+	+++	−	++	−	+
				5	+++	+++	+++	++	+	+++
YEN	22	2	BOS	3	−	−	−	+	−	−
				5	+	+++	−	−	−	++
			BON	3	−	−	−	−	−	+
				5	++	+++	+	++	+	+++
		3	BOS	3	−	+	−	+	−	−
				5	−	+	−	++	−	−
			BON	3	−	+	−	+	−	+
				5	+++	+++	++	+++	+++	+++

[a] *P. putida, P. morganii, E. cloacae, S. liquefaciens, E. agglomerans*, and *C. freundii*; background count, 2×10^{6}/g.
[b,c,d] See Table 1.

according to results of the third and fourth experiments. In the fifth experiment (Table 5) the results improved, but the system again failed in the experiment that followed (Table 6).

The last inoculated food experiment was designed to study two cold preenrichment media, PSB at 4°C and YER at 4 and 10°C, and to determine the optimum incubation time required for BOS when used with these preenrichments (Table 7). Nine different strains of *Y. enterocoli-*

TABLE 5. Recovery of *Y. enterocolitica* from turkey-chicken stew containing an artificial background flora[a]

Preenrichment			Selective enrichment (22°C)		Recovery of *Y. enterocolitica*[b] (serotype)					
					E736 (O:21)		E759 (O:4,32)		E768 (O:1,2,3)	
Medium	Temp (°C)	Time (days)	Medium	Time (days)	8.6×10^{1}[c]	8.6×10^{3}	7.8×10^{1}	7.8×10^{3}	1.2×10^{2}	1.2×10^{4}
YER	10	3	BOS	3	+++	+++	+++	+++	−	+++
									(++4d)	
			MRB	3	+	+	−	+	+	++
YEN	22	2	BOS	3	−	++	+	++	+	+++
				4	−	ND[d]	++	ND	++	ND
MRB	22	6	None		+	++	−	+	+++	+++
PSB	4	14	None		−	−	−	+	−	−
			BOS	3	+++	+++	+++	+++	+	++
			MRB	3	−	+	−	+	−	++

[a] *E. agglomerans, E. coli, A. hydrophila, C. freundii, S. liquefaciens*, and *P. aeruginosa*; background count, 1.8×10^{5}/g.
[b,c] See Table 1.
[d] ND, not done.

TABLE 6. Recovery of *Y. enterocolitica* from turkey-chicken stew containing an artificial background flora[a]

Preenrichment			Selective enrichment (22°C)		Recovery of *Y. enterocolitica*[b] (serotype)					
Medium	Temp (°C)	Time (days)	Medium	Time (days)	E736 (O:21)		E759 (O:4,32)		E768 (O:1,2,3)	
					9.0×10^{1}[c]	9.0×10^{3}	7.9×10^{1}	7.9×10^{3}	1.2×10^{2}	1.2×10^{4}
YER	10	3	BOS	3	+	+++	++	+++	− (+++7d)	+
			MRB	3	−	+	−	−	+	++
YEN	22	3	BOS	3	−	−	−	−	−	+
				4	−	−	−	++	−	−
MRB	22	6	None		+	++	−	+	+++	+++
PSB	4	14	None		−	−	−	+++	−	++
			BOS	3	+	+++	+	+++	−	+
				5	ND[d]	ND	+++	ND	+++	+++
			MRB	3	+	++	+	++	++	+++

[a] *K. pneumoniae, C. freundii, P. fluorescens, E. aerogenes, P. morganii,* and *S. liquefaciens*; background count, 1.9×10^{5}/g.
[b,c] See Table 1.
[d] ND, not done.

tica representing six serotypes were introduced individually at one cell density, about 10^{2}/g, along with a background composed of six gram-negative bacteria at a density of 1.8×10^{6}/g. The use of YER instead of PSB at 4°C reduced the time required for cold enrichment from 14 to 9 days for recovery of all strains. A shorter incubation time for YER of 6 days increased the incubation time for BOS to achieve the same result. The slowest growers in BOS were the two strains of serotype O:5,27. Incubation of YER for 14 days provided recovery of all strains from BOS after only 3 days, which was not the case when PSB was used as the preenrichment. The use of BOS after cold enrichment with YER tended to reduce the amount of background flora on CIN isolation agar.

The results demonstrate that the common cold enrichment procedure with PSB at 4°C for 14 days is not a productive system for recovery of *Y. enterocolitica* from foods. The problem lies primarily in separation of *Y. enterocolitica* from background organisms that have also grown to high numbers and are not inhibited on the selective agar media used for isolation. Although CIN agar is considerably more selective than other enteric media used for isolation of *Y. enterocolitica* (19), it is still unable to completely suppress the background flora present after cold enrichment. Aulisio et al. (2) described a technique for

TABLE 7. Recovery of *Y. enterocolitica* from turkey-chicken stew with an artificial background flora[a]

Preenrichment			Time (days) for recovery of *Y. enterocolitica*[b] (serotype) from BOS at 22°C								
Medium	Temp (°C)	Time (days)	E752 (O:3) 1.1×10^{2}[c]	E742 (O:3) 1.2×10^{2}	E662 (O:8) 6.7×10^{1}	E665 (O:8) 8.2×10^{1}	E758 (O:5,27) 9.0×10^{1}	E654 (O:5,27) 1.0×10^{2}	E736 (O:21) 9.0×10^{1}	E759 (O:4,32) 1.2×10^{2}	E768 (O:1,2,3) 1.1×10^{2}
YER	10	2	4 (++)	6 (++)	3 (+)	3 (+++)	6 (+)	−	3 (++)	3 (+++)	5 (++)
		3	4 (++)	4 (++)	3 (+)	3 (+++)	5 (+)	4 (+)	3 (+)	3 (++)	4 (+)
YER	4	4	4 (++)	4 (+)	4 (+)	3 (++)	−	−	4 (++)	3 (++)	4 (+)
		6	4 (++)	4 (++)	4 (+)	3 (+++)	6 (+++)	7 (+)	3 (+)	3 (++)	5 (+++)
		9	3 (+)	3 (+)	3 (+)	3 (+++)	4 (+)	5 (+)	3 (+++)	3 (+++)	3 (+)
		14	3 (++)	3 (++)	3 (+++)	3 (+++)	3 (++)	3 (+)	3 (+++)	3 (+++)	3 (++)
PSB[d]	4	14	−	−	−	−	+	−	−	−	−
PSB	4	4	4 (+)	4 (+)	5 (+)	3 (+)	7 (+)	−	4 (+)	4 (++)	4 (+)
		7	4 (+)	4 (+)	3 (+)	3 (++)	−	−	4 (+)	4 (+++)	4 (+)
		10	4 (+)	4 (+)	4 (+)	3 (+++)	4 (+)	7 (+)	3 (+++)	4 (++)	4 (+)
		14	3 (+)	4 (+)	3 (+)	3 (+++)	5 (+++)	4 (+)	3 (+)	3 (++)	4 (+)

[a] *P. putida, P. morganii, E. cloacae, S. liquefaciens, E. agglomerans,* and *C. freundii*; background count, 1.8×10^{6}/g.
[b,c] See Table 1. Cultures were examined daily from 3 to 7 days.
[d] PSB direct without selective enrichment.

TABLE 8. Tolerance of *Y. enterocolitica* and other gram-negative bacteria for potassium hydroxide[a]

Test organism (serotype)	Count per ml								
	Initial	32°C			22°C		10°C		
		15 min	30 min	45 min	15 min	30 min	45 min	15 min	30 min	45 min
Y. enterocolitica (O:8)	1.4×10^5	5.5×10^3	$<10^3$	$<10^3$	4.5×10^4	3.1×10^4	3.0×10^4	9.8×10^4	8.1×10^4	5.7×10^4
Y. enterocolitica (O:5,27)	1.7×10^5	3.3×10^4	1.5×10^3	$<10^3$	1.0×10^5	7.4×10^4	5.3×10^4	1.2×10^5	1.2×10^5	1.2×10^5
Y. enterocolitica (O:5,27)	1.8×10^5	2.0×10^3	$<10^3$	$<10^3$	9.1×10^4	5.4×10^4	3.9×10^4	1.1×10^5	1.0×10^5	9.2×10^4
Y. enterocolitica (O:3)	2.1×10^5	$<10^3$	$<10^3$	$<10^3$	6.0×10^3	$<10^3$	$<10^3$	6.6×10^4	2.6×10^4	1.4×10^4
P. fluorescens	4.7×10^4	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	5.0×10^2	$<10^3$	$<10^3$
P. fruendii	2.0×10^5	$<10^3$	$<10^3$	$<10^3$	4.0×10^3	$<10^3$	$<10^3$	9.2×10^4	3.6×10^4	4.5×10^3
S. liquefaciens	2.0×10^5	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	1.0×10^4	$<10^3$	$<10^3$

[a] A total of 100 μl of 20% KOH was added per 10 ml of cell suspension in YER medium; pH after addition, 11.4.

achieving separation of *Y. enterocolitica* from background by briefly treating the enrichment with KOH (0.5%) before streaking onto the isolation agar. A study of KOH tolerance by four strains of *Y. enterocolitica* and three species of other gram-negative bacteria in YER medium at three temperatures showed that *Y. enterocolitica* was indeed more resistant than the other organisms (Table 8). However, one strain of *Y. enterocolitica* (serotype O:3) showed a sensitivity not too different from that of *C. freundii*. KOH treatment at the same concentration was then applied to the YER broth enrichment of a sample of celery inoculated with three strains of *Y. enterocolitica*. The purpose was to determine whether KOH treatment of the preenrichment would give a competitive advantage to *Y. enterocolitica* in the selective enrichment. The exposure times were 1 min at 32°C, 5 min at 22°C, and 15 min at 10°C. Recovery was compared after selective enrichment in BOS; no improvement was observed over BOS inoculated with YER without KOH treatment. There was also no apparent change in the relative amount of background growth on the isolation agar.

Recovery from pork. Eight enrichment systems and direct isolation were compared for their relative ability to recover *Y. enterocolitica* from 10 retail pork samples (four ground meat and six tongues) plus 20 tongues taken from freshly slaughtered swine. YER was incubated at 10°C for 3 days and at 4°C for 9 days. PSB was incubated at 4°C for 14 days. YEN was incubated at 22°C for 2 days. MRB was incubated at 22°C for 6 days when used directly and for 4 days when used after PSB preenrichment. BOS and BON were always incubated at 22°C for 5 days. The results presented in Table 9 show that four enrichment systems were essentially equivalent for recovery of all species of *Yersinia* from retail pork samples: YER 10°C–BON, YER 10°C–BOS, YER 4°C—BOS, and PSB–BOS. All 10 samples were positive for *Y. enterocolitica* by the YER 10°C–BON enrichment procedure. YEN–BON showed nearly the same number of isolations of *Y. enterocolitica*, but fewer of the other species. The other enrichment systems, included as reference methods for comparison, were far inferior. The necessity of enrichment was clearly demonstrated by the low recovery obtained by direct streaking.

Serotyping of selected isolates of *Y. enterocolitica* obtained from 10 retail pork samples showed that there were at least eight different serotypes and nontypable strains present. Only one isolate of serotype O:3 was obtained, and it was recovered by the PSB-MRB method. The YEN-BON procedure recovered the greatest variety of serotypes.

TABLE 9. Productivity of different enrichment methods for recovery of *Yersinia* spp. from 10 retail pork samples

Enrichment method	No. of samples positive for:					Serotypes of *Y. enterocolitica* (no. of positive samples)
	Y. enterocolitica	*Y. frederiksenii*	*Y. kristensenii*	*Y. intermedia*	Atypical	
None (direct)	1	0	0	0	1	NT[a] (1)
PSB (4°C, 14 days)	2	1	4	3	1	O:7,13 (1); O:6,30 (1)
MRB (22°C, 6 days)	0	0	0	1	0	
PSB (4°C, 14 days), MRB (22°C, 4 days)	1	1	0	0	0	O:3 (1)
YER (10°C, 3 days), BOS (22°C, 5 days)	8	2	4	9	2	NT (4); O:7,13 (1); O:7,8 (1); O:6,30 (1); O:41,42 (2)
YER (10°C, 3 days), BON (22°C, 5 days)	10	0	1	7	3	NT (6); O:5 (4); O:41,42 (1); O:6,30 (2)
YER (4°C, 9 days), BOS (22°C, 5 days)	9	1	5	8	3	NT (4); 0:7,8 (1); O:41,42 (1); O:6,30 (3)
PSB (4°C, 14 days), BOS (22°C, 5 days)	7	1	3	7	2	NT (3); O:7,13 (1); O:6,30 (4)
YEN (22°C, 2 days), BON (22°C, 5 days)	7	0	0	1	2	NT (1); O:7,8 (1); O:7,13 (1); O:5 (2); O:36 (1); O:41,42 (1); O:21 (1); O:6,30 (2)

[a] NT, Nontypable.

Eighteen of the isolates obtained from retail pork samples could not be placed into any of the four species of *Yersinia*. The biochemical profiles for these atypical cultures are shown in Table 10.

The results obtained with fresh pork tongues are likely influenced by two differences in flora compared with retail pork samples: (i) the background flora is less in number and variety, and (ii) the predominant type of *Y. enterocolitica* is serotype O:3. No other species of *Yersinia* was isolated from fresh pork tongues. The different background improves recovery by PSB cold enrichment alone (60%), so that a selective enrichment (BOS) after PSB showed only a slight improvement (70%) (Table 11). Because the common serotype is O:3, which grows well in MRB, this enrichment showed good recovery after PSB (70%). However, MRB used directly showed very poor recovery (10%), possibly because this medium does not always support growth from a low inoculum. BOS used after

TABLE 10. Biochemical profiles[a] for 18 atypical isolates of *Yersinia* obtained from retail pork products

Profile no.	No. of isolates	KIA, 35°C	Urea, 35°C	Salicin, 35°C	Esculin, 22°C	ONPG,[b] 35°C	Maltose, 35°C	ONPG, 22°C	Motility, 22°C	Ornithine, 22°C	Sucrose, 22°C	Voges-Proskauer, 22°C	Rhamnose, 22°C	Raffinose, 22°C	Mellibiose, 22°C	α-Methylglucoside, 22°C	Citrate, 22°C	Lecithinase, 22°C	Indole, 30°C
1	2	A/A	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	+
2	1	K/A	+	+	−	+	+	+	+	+	−	−	−	−	−	−	+	−	−
3	4	K/A	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	−	+
4	2	K/A	+	+	+	+	+	+	−	+	+	+	−	+	+	+	−	−	+
5	1	K/A	+	+	+	−	+	−	+	+	+	+	+	−	−	+	+	+	+
6	1	K/A	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	−	+
7	1	K/A	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	+
8	1	A/A	+	+	+	+	+	+	−	+	+	+	−	+	−	−	−	+	+
9	1	K/A	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
10	2	A/A	−	+	+	+	−	+	−	+	+	+	−	+	−	−	−	+	+
11	1	K/A	+	+	−	+	+	+	+	−	−	−	−	−	−	−	+	−	−
12	1	K/A	+	−	−	+	+	+	+	+	+	−	−	+	+	−	+	−	−

[a] All isolates were negative for H_2S and gas on KIA; negative for motility, dulcitol, and lysine decarboxylase at 35°C; and positive for mannitol at 35°C and xylose at 22°C.

[b] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.

TABLE 11. Productivity of different enrichment methods for recovery of *Y. enterocolitica* from 20 fresh pork tongues

Enrichment method	No. of positive samples (%)	Serotypes isolated (no. of positive samples)
None (direct)	3 (15)	O:3 (3)
PSB (4°C, 14 days)	12 (60)	O:3 (8); O:22 (4); O:15 (2); O:46 (1); NT[a] (5)
MRB (22°C, 6 days)	2 (10)	O:3 (2)
PSB (4°C, 14 days), MRB (22°C, 4 days)	14 (70)	O:3 (12); O:15 (1); NT (1)
YER (10°C, 3 days), BOS (22°C, 5 days)	15 (75)	O:3 (4); O:7,8 (1); O:22 (3); O:5 (6); NT (3)
YER (10°C, 3 days), BON (22°C, 5 days)	11 (55)	O:3 (10); O:7,8 (1); O:5 (3); NT (2)
YER (4°C, 9 days), BOS (22°C, 5 days)	17 (85)	O:3 (8); O:15 (3); O:22 (2); NT (11)
PSB (4°C, 14 days), BOS (22°C, 5 days)	14 (70)	O:3 (6); O:15 (3); O:22 (2); NT (8)
YEN (22°C, 2 days), BON (22°C, 5 days)	8 (40)	O:3 (6); O:5 (1); O:6 (1)

[a] NT, Nontypable.

YER at 4°C gave the best recovery (85%), followed by BOS after YER at 10°C (75%). BON with either YER at 10°C (55%) or YEN (40%) was not highly productive, confirming observations with pure cultures that some strains of serotype O:3 are salt sensitive (unpublished data).

Serotyping of selected isolates from the 20 fresh pork tongues indicated that at least seven different types and nontypable strains were distributed among the samples. The PSB and YER 10°C–BOS enrichment systems recovered the greatest variety of serotypes, whereas the PSB-MRB system recovered the greatest number of serotype O:3.

Performance of CIN agar. A total of 461 colonies were selected from CIN agar for identification. Selection was based on the typical colonial appearance of *Y. enterocolitica* on this medium, i.e., a colony having a red "bullseye," usually very dark and sharply delineated, surrounded by a transparent zone that varies in radius, with the edge of the colony sometimes entire and other times irregular (19). A total of 425 colonies (92.2%) were identified as *Yersinia*. The remaining 36 isolates were distributed as follows: 25 *Enterobacter agglomerans*, 4 *S. liquefaciens*, 3 *Serratia marcescens*, 2 *Proteus vulgaris*, 1 *Alcaligenes* sp., and 1 API group 2. Twenty-two of the isolates of *E. agglomerans* came from the PSB enrichment, and the remaining three from the PSB-MRB system. This indicates that this organism, which is more frequently confused with *Y. enterocolitica* on CIN agar than any other, is less likely to come through the new enrichment systems described here, i.e., YER and BOS.

Devenish and Schiemann (6a) have reported that *Y. enterocolitica* grows with a typical colonial appearance on CIN agar that is seldom presented by other gram-negative bacteria on this medium, and that these other gram-negative organisms could be differentiated by the use of two biochemicals, a KIA plus a urea slant. Of the 36 presumptive colonies selected from CIN agar in this study that were not *Y. enterocolitica*, all could be separated by these two biochemicals, except two isolates of *P. vulgaris*, which, like *Y. enterocolitica*, gave alkaline-acid without gas reactions on the KIA slant and were urease positive. There were, however, another 31 cultures that were typical on KIA but were urea negative and proved to be *Y. enterocolitica* (30 cultures) or *Y. intermedia* (1 culture) on further testing. Twenty of these cultures were isolated from fresh pork tongues, were either serotype O:22 or nontypable, and were unusual in being nonmotile at 22°C. They were otherwise typical of *Y. enterocolitica*, being positive for indole, xylose, lecithinase, sucrose, Voges-Proskauer test, salicin, and esculin and negative for rhamnose, raffinose, melibiose, α-methylglucoside, and citrate.

DISCUSSION

The use of a selective enrichment broth after preenrichment has several advantages over single-step techniques for the recovery of specific bacteria from foods with a mixed flora. The preenrichment medium can be less selective, allowing for growth of small inocula and repair of injured or stressed cells. The selective enrichment will not be overwhelmed by drastic changes in composition with addition of food. The selective enrichment can be highly selective since the inocula will usually be greater in size and consist of healthy cells after growth in the preenrichment broth.

BOS enrichment medium was developed after extensive studies on minimal growth requirements for *Y. enterocolitica* and tolerance of the organism for various selective agents in a low-nutrient medium. Most of these developmental studies were completed with strains of serotype O:8 because it has been one of the most difficult to recover from foods (12, 20). Consequently, the outcome was a medium that provided for better growth of serotype O:8 strains than many

others. Good growth can be obtained in BOS with an inoculum of about 100 cells within 3 days at 22°C. Strains of serotype O:5,27 have the greatest difficulty in BOS medium, requiring at least 5 days for good growth. For some unknown reason, the addition of 2.5% NaCl to BOS (designated BON broth) improves the growth of serotype O:5,27 strains added as pure cultures while also increasing the selectivity of the broth. Unfortunately, some strains of other serotypes, including O:3, were found to be salt sensitive in this medium.

Modified Rappaport broth is a highly selective enrichment medium even without the inclusion of carbenicillin, which was found inhibitory for some strains of serotype O:3. It is, however, only suitable for recovery of certain serotypes of *Y. enterocolitica*, particularly O:3 and O:9. Wauters Ph.D. thesis found that MRB at room temperature was far superior to cold enrichment for recovery of *Y. enterocolitica* serotype O:3 from swabs of pork tongues when using a very light inoculum. In the inoculated food studies reported herein MRB used directly at a food/broth ratio of 1:10 worked well for recovery of serotype O:3 strains (Table 2) and also for recovery of serotypes O:21 and O:4,32 (Tables 5 and 6). However, the performance of MRB after cold enrichment with either PSB or YER was erratic (Tables 2, 5, and 6). In contrast, with pork tongues MRB used for direct enrichment performed poorly, but extremely well after PSB for recovery of serotype O:3 (Table 11). In this latter case the MRB broth was incubated for 4 days instead of the 3 days used in the food inoculation studies. In a previous study of MRB with cold enrichment, Schiemann and Toma (23) found that this two-step system improved recovery of *Y. enterocolitica* from raw milk.

Y. enterocolitica is obviously a poor competitor in any system with a mixed bacterial flora, but can apparently compete better at lower temperatures. Early studies attempted to use higher incubation temperatures for both preenrichment and selective enrichment, but were never completely successful in that certain other gram-negative bacteria, particularly *S. liquefaciens*, would consistently outgrow and destroy small numbers of *Y. enterocolitica*. The major disadvantage of low-temperature enrichment is, of course, the longer time that is required. The enrichment methods described here were not dramatic in reducing this time requirement. The use of YER for preenrichment over PSB reduced the time at 4°C from 14 to 9 days. Incubation of YER at 10°C reduced the preenrichment time to 3 days, but recovery was not always equivalent to preenrichment at 4°C. Strains of serotype O:8 grew very well in BOS and could be recovered after only 3 days of incubation after preenrichment in YER at 10°C for 3 days. This held the total time for enrichment to 6 days for this particular serotype. The addition of NaCl to BOS (BON broth) reduced the time for growth of serotype O:5,27 strains and improved recovery of certain other types on occasion (Table 4). The use of NaCl in YER (YEN broth) allowed preenrichment at 22°C for 2 days, reducing the total enrichment time to 7 days. However, if one wishes to choose a single enrichment system that is more likely to recover all types of *Y. enterocolitica*, it would be preenrichment at 4°C with either PSB for 14 days or YER for 9 days followed by selective enrichment with BOS at 22°C for 5 days. These two systems increase the total time for enrichment to either 19 or 14 days.

An obvious difficulty with the design of enrichment systems for recovery of *Y. enterocolitica* from foods is the heterogeneity of the organisms included in the genus *Yersinia*. Different biotypes and serotypes of *Yersinia* also differ physiologically. The problem is somewhat reduced by the recognition that only organisms now accepted as *Y. enterocolitica* (3) will include forms that are virulent according to laboratory models and epidemiological data, and that related species and atypical forms that are commonly found in foods, but rarely in humans, are very likely of no medical importance (22; unpublished data). It is still difficult to select a single enrichment system that will be equally efficient for recovery of all pathogenic forms of *Y. enterocolitica*. Consequently, one must consider the type of *Y. enterocolitica* sought when selecting an enrichment system, and obviously the use of more than one system will provide the greatest recovery potential.

Examination of the serotypes isolated by certain enrichment procedures applied to retail pork samples and pork tongues indicates that the rate of positive samples is not the only nor necessarily the best criterion for judging the performance of an enrichment method. With respect to the retail pork samples, the YER 10°C–BON method gave the greatest number of positive samples, but the YEN-BON method recovered the largest variety of serotypes. The PSB-MRB method found only one sample positive, but it also provided the only isolate of serotype O:3. The best procedure for isolation of serotype O:3 from pork tongues was PSB-MRB (12 positive samples), but YER 4°C–BOS gave the highest number of positive samples, and YER 10°C–BOS and PSB gave the greatest variety of serotypes. YER 4°C–BOS showed the highest rate of positive samples (85%), but recovered serotype O:3 from only 8 samples compared with 12 for the PSB-MRB method. The YER 4°C–BOS procedure found 11 samples pos-

itive for nontypable strains, the highest for any procedure. This suggests that enrichment procedures that have a lower selectivity permit recovery of a wider spectrum of types of *Yersinia*, but, because of competitive interactions, are more likely to miss specific types. The results are also influenced by the methodology used for selection of isolates which depends on individual colonies on the isolation agar that in turn represent the type(s) that has been the strongest competitor and reached the highest number in the enrichment medium.

The isolation of the common human serotype O:3 (24) from swine has been frequently reported (18, 31). The isolation rate for serotype O:3 from fresh pork tongues was 60%, which compares closely to that previously reported for pork tongues from retail outlets by Schiemann (21) and by Wauters (30). None of the other serotypes obtained in this study represents an important human pathogen (22). It is unfortunate that the new enrichment methods presented here could not be evaluated with natural foods containing other important human serotypes such as O:8 and O:5,27, but reservoirs or foods containing these types with any consistent frequency have not been identified. Nevertheless, the experimental results obtained with inoculated foods as well as natural foods indicate that the selective enrichment BOS is an improved medium for the isolation of a wide spectrum of *Y. enterocolitica* types, including those that are pathogenic for humans.

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